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Cell Mimicry as a Bottom-up Strategy for Hierarchical Engineering of Nature-Inspired Entities

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Abstract

Artificial biology is an emerging concept that aims to design and engineer the structure and function of natural cells, organelles, or biomolecules with a combination of biological and abiotic building blocks. Cell mimicry focuses on concepts that have the potential to be integrated with mammalian cells and tissue. In this feature article, we will emphasize the advancements in the past 3-4 years (2017-present) that are dedicated to artificial enzymes, artificial organelles, and artificial mammalian cells. Each aspect will be briefly introduced, followed by highlighting efforts that considered key properties of the different mimics. Finally, the current challenges and opportunities will be outlined.

Graphical/Visual Abstract and Caption

Artificial enzymes, artificial organelles, and artificial cells as the three core building blocks in cell mimicry.
1. INTRODUCTION

Artificial biology envisions employing the recent advances in chemistry, biology, nano- and material-science to equip cells with selected functions following a bottom-up strategy. However, it is an undeniable fact that even the simplest unicellular organisms are exceedingly complex with the current cognition of biology. Numerous efforts were devoted to design inanimate entities with single or multiple cellular functionalities and integrate them into a sophisticated cell-like machinery on the way to fulfill the ultimate goal of mimicking natural counterparts. Mimicking a cell and its building blocks with semi-synthetic materials, that is, molecules/assemblies that consist of a combination of natural and synthetic materials, requires the consideration of two fundamental questions: what does a cell do and what does a cell look like. Simply speaking, for the former, one of the basic functions of a cell is to catalyze a variety of biochemical reactions for the metabolic pathways to work properly in a living organism. For the latter, it could be divided into two relevant subtopics: structure of the cell per se and structure of the subcellular entities, namely, the organelles. That leads to the three essential building blocks of cell mimicry: artificial enzymes (AEs), artificial organelles (AOs), and artificial cells (ACs). Specifically, AEs aim to mimic the catalytic activity of intracellular enzymes that are responsible for the diverse biochemical transformations; AOs are subcellular compartments devised to perform specialized functions. Their semi-permeability allows for the transportation and exchange of certain molecules through the porous membranes; Lastly, ACs are an integration of the above compartments constructed in a hierarchical and controllable manner without losing their functionalities.

This focus article will highlight the most recent developments of these three core building blocks of cell mimicry focusing on the past 3-4 years (Scheme 1). Each section will provide a short introduction followed by selected examples. First, we will discuss the different types of AEs, including artificial metalloenzymes, inorganic nanoparticles (nanozymes), and the use of supramolecular scaffolds before outlining aspects on substrate specificity and the target applications of AEs. Next, we will elaborate about AOs considering their types and core properties, such as cytosolic placement, permeability, and activity. Finally, the recent developments in bottom-up assembly of ACs will be outlined. Key aspects for their natural counterparts, such as compartmentalization, encapsulated catalysis, communication, and energy transduction will be specifically pointed out.
2. ARTIFICIAL ENZYMES

Enzymes are biocatalysts that are responsible for accelerating virtually all the biochemical reactions taking place in living organisms with excellent efficiency and selectivity. (Wolfenden et al., 2001; Benkovic et al., 2003) Over time, natural enzymes have evolved to exhibit highly sophisticated three-dimensional structures, which usually consist of a catalytic site located close to one or more binding sites for substrates, surrounded by a globular protein scaffold to maintain the orientation and dynamics of the active site. (Agarwal 2006) Albeit natural enzymes usually show superior catalytic efficiency and substrate specificity under mild conditions, some of their intrinsic drawbacks, such as low stability in

SCHEME 1 Cartoon of a mammalian cell containing artificial enzymes (AEs), artificial organelles (AOs) as well as natural organelles. Artificial biology aims to mimic functional and structural aspects of natural cells ranging from enzymes, organelles over the whole cell. (a) Examples of AEs (metalloenzymes (i), cyclodextrin as an example of a self-assembled supramolecular scaffold (ii) and inorganic particles with polymeric imprinting(iii)). (b) AOs (liposomes with encapsulated enzymes (i), synthetic hollow mesoporous nanospheres (ii), polymersome with membrane pores that allow small molecules to transport across the membrane (iii) and a micelle with an active enzymatic core(iv)). (c) ACs (giant vesicle with natural organelles encapsulated (i), hydrogel bead with encapsulated liposomes with biocatalytic activity (ii) are shown schematically). Typical size ranges are indicated in the scale bar at the bottom.
ambient environment, difficulty in preparation and purification, and in many cases, the need for a specific cofactor or coenzyme, severely limited their practical applications. AEs are thus created to simulate the catalytic complexity and function of their natural counterparts, yet circumvent the abovementioned shortcomings. With elaborate design, AEs are not only capable of rival in catalytic performance but also endowed with additional valuable merits, such as high stability, tailor-made activity, and easy preparation that their natural equivalents do not possess. Constructing AEs is undoubtedly a fundamental yet pivotal step toward the path of engineering cell mimicry. Over the years, scientists have explored the possibilities of applying different scaffolds to pursue the goal of finding materials with enzyme-like activities, and the efforts are rewarding. To date, a plethora of natural enzymes, such as peroxidase, oxidase, catalase, hydrolase, hydrogenase, carbonic anhydrase superoxide dismutase, laccase, and so on, have found their artificial substitutes, even though sometimes compromised with insufficient catalytic activity and poor substrate specificity. Previously, numerous excellent reviews with regard to AEs were published concentrating on different aspects, which are recommended for readers in order to gain a more in-depth understanding of this rapidly expanding realm. (Dong et al., 2012; Kuah et al., 2016; Wu et al., 2019) In this section, we mainly highlight the most recent progress of AEs from the past 3-4 years.

2.1 Artificial metalloenzymes

Artificial metalloenzymes (ArMs), which structurally resemble natural metalloenzymes with a cofactor immobilized in a host protein scaffold, are a complement to the natural counterparts replaced with a variety of synthetic unnatural amino acid sequences and/or abiotic cofactors, assembled covalently or noncovalently. (Lin 2017; Schwizer et al., 2018; Yu et al., 2018) While the choices of amino acids and cofactors are limited for naturally occurring metalloenzymes, ArMs use synthetic building blocks to combine the features of both enzymatic catalysis and homogeneous catalysis, which significantly broadens the scope of catalytic reactions that are not present in nature. (Jeschek et al., 2016; Key et al., 2016) However, the development of ArMs had been stagnant for a long period since the pioneering work presented by Wilson and Whitesides in 1978. (Wilson et al., 1978) The main hurdles were the difficulties in the synthesis of organometallics and protein scaffolds, as well as the precise control of anchoring the metallocofactors within the second coordination environment of the protein. In the past few years, the vast progress in organometallic synthesis and prominent protein scaffolds prepared with directed evolution, such as hemeprotein, (Hayashi et al., 2018) nitrobindin, (Grimm et al., 2018) albumin, (Eda et al., 2019) and CeuE(Raines et al., 2018) as well as robust anchoring strategies, for example, biotin-streptavidin technology, (Mallin et al., 2016; Liang et al., 2019) have been well-established, which promoted ArMs to be widely utilized to catalyze a wide range of abiotic reactions, such as cyclopropanation, (Reynolds et al., 2017; Villarino et al., 2018; Yang et al., 2018) C-H activation, (Gu et al., 2019) Diels-Alder cycloaddition, (Ghattas et al., 2018) oxidation, (Doble et al., 2018) etc. One of the prominent examples was reported by Vong and coworkers. (Vong et al., 2019) They prepared a novel ArM with a ruthenium-based active site anchored to an albumin scaffold, which was nonfluorescent due to an intramolecular Förster resonance energy transfer (FRET) interaction (Figure 1ai). In the presence of phytohormone ethylene, a ruthenium-catalyzed cross metathesis occurred, which interrupted the FRET process and restored fluorescence of the fluorophore. Such a fluorescence turn-on process could be utilized to detect a trace amount of ethylene in both fruits and plant leaves (Figure 1aii).
2.2 Inorganic nanoparticles

Inorganic nanomaterials, such as metal, metal oxide, and carbon allotrope-based nanoparticles (NPs) contributed a great extent to the AEs family because of their facile preparation, high stability, high surface area to volume ratio, and easy surface functionalization. Ever since the first unprecedented report in which Gao and co-workers discovered the intrinsic peroxidase-like activity of Fe$_3$O$_4$ magnetic NPs,(Gao et al., 2007) scientists have been inspired to explore the potential enzyme-mimicking competence of various other NPs promoted by the rapid development of synthesis and characterization in this field.(Wei et al., 2013; Huang et al., 2019; Wang et al., 2019) In recent years, gold (Hu et al., 2017; Hu et al., 2018; Oh et al., 2018), silver (Bagheri et al., 2018; Karim et al., 2018), iron (Mumtaz et al., 2017; Bhattacharjee et al., 2018), platinum (Deng et al., 2017; Jin et al., 2017; Zhang et al., 2018), manganese (Liu et al., 2017; Yao et al., 2018), cerium (Liu et al., 2017; Meng et al., 2019), graphene (Nirala et al., 2017; Wang et al., 2017; Hu et al., 2018) and their oxides-based NPs were widely explored to emulate an assortment of natural enzymes. Their catalytic activity is mainly affected by size, morphology, composition, surface modification, and ambient environment (e.g. light, pH, redox conditions, and levels of oxygenation). More recently, hybrid NPs, either homogeneously alloyed or heterostructured, integrating the merits of two or even more metals, have aroused great interests in application as multifunctional AEs to catalyze a cascade reaction with enhanced performance and enriched functionalities, such as magnetism, surface-enhanced Raman scattering, luminescence, near-infrared absorption, etc.(Wang et al., 2017; Wang et al., 2018; Mu et al., 2019) Other than that, immobilizing NPs on a porous substrate, such as metal-organic frameworks (MOFs)(Hu et al., 2017; Huang et al., 2017; Zhang et al., 2018) and silica,(Huo et al., 2017; Gao et al., 2019) has been a frequently adopted technique to inhibit the aggregation of the NPs and improve their catalytic performance.

2.3 Supramolecular scaffolds

Complex, hierarchical yet dynamic supramolecular scaffolds are desirable frameworks for the development of novel AE models. It is well known that the unique catalytic microenvironment of the active sites in natural enzymes is crucial for substrate binding and subsequent catalytic cascade reactions, which is a direct result of supramolecular interactions, e.g., folding and self-assembly of the proteins. In addition to the above-mentioned ArMs, compounds with macrocyclic cavities, either natural or synthetic, which could form inclusion complex with substrates via non-covalent interactions, such as cyclodextrin,(Wang et al., 2017; Wang et al., 2017) cucurbituril,(Kubota et al., 2018) crown ether,(Ning et al., 2018) etc. were widely considered to provide this microenvironment to confine substrates and allow for more effective and selective catalysis. Except for these macromolecular models, self-assembled nanocompartments, such as molecular cages,(Roy et al., 2017; Marti-Centelles et al., 2018; Nurttila et al., 2019) micelles,(Dou et al., 2017; Arifuzzaman et al., 2018) vesicles,(Blackman et al., 2018; Fuhrmann et al., 2018) dendrimers,(Li et al., 2018; Morshed et al., 2019) and core-shell particles,(Keller et al., 2017) which could immobilize either natural or AEs and generate a protective and substrate-dependent environment for the active molecules and improve the catalytic efficiency and selectivity were also extensively evaluated. We recently demonstrated the preparation of micelles loaded with a salen-manganese complex (EUK), which exhibited catalase-like activity (Figure 1bii and Figure 1biii).(Ade et al., 2019). Intracellular activity in HepG2 cells was illustrated by scavenging reactive oxygen species (ROS) and improving cell viability (Figure 1biii).
2.4 Substrate specificity

The two most fundamental features of enzymes are catalytic activity and substrate specificity. The former remains the mostly addressed aspect, while the latter is rarely considered and in many cases, a matter of serendipity for AEs. In nature, the specific three-dimensional conformation of the protein wrapped around the active site and the complementary affinity between the binding site and the substrate work collectively to screen for substrates with high chemoselectivity, regioselectivity, and stereospecificity. Conversely, AEs generally show poor substrate specificity stemming from the absence of selective substrate binding sites. In addition to directed evolution of proteins and microenvironment-related considerations, another potential method to improve substrate specificity is to attach substrate-recognizing natural receptors, for example, aptamers,(Sharma et al., 2014; Flanagan et al., 2018) to the AEs. Molecular imprinting, which introduces robust and cost-effective polymeric template sites onto the surface of AEs, is an alternative to the natural receptors.(Cieplak et al., 2016; Zhang et al., 2017) An interesting example in this context was reported by Zhang et al.(Zhang et al., 2017) They demonstrated that the substrate specificity could be improved by the growth of molecularly imprinted polymers (MIPs) on the surface of Fe₃O₄ NPs to create substrate-binding pockets (Figure 1ci). Compared with non-coated NPs, nearly 100-fold higher specificity was achieved for the selected substrate 3,3′,5,5′-tetramethylbenzidine (TMB) over a comparative substrate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS) (Figure 1cii). In a follow-up effort, they argued that the molecular imprinting method could also accelerate the catalytic reaction.(Zhang et al., 2019) Bagheri and co-workers harnessed a similar procedure to molecularly imprint Ag NPs/Zn-based metal-organic framework nanocomposite to fulfil the selective detection of patulin (Bagheri et al., 2018).

2.5 Applications

As a low-cost alternative to natural enzymes, AEs have found broad applications in biomedical realm. Various vital biomolecules, such as glucose,(Hu et al., 2017; Han et al., 2018; Zhang et al., 2018) glutathione,(Liu et al., 2017) lactate,(Deng et al., 2017) dopamine,(Yan et al., 2018) heparin,(Cheng et al., 2017; Hu et al., 2018) prostate-specific antigen,(Ye et al., 2017) etc. could be detected in cell culture and in animal models with high sensitivity using AEs. In addition to sensing applications, the feasibility of applying AEs as a medical intervention for therapeutic purposes has also drawn scientist’s attention, typically focusing on elevating ROS levels to induce cell apoptosis and necrosis.(Liu et al., 2018; Wang et al., 2018; Liu et al., 2019) For instance, Fan and co-workers developed nitrogen-doped porous carbon nanospheres (N-PCNSs) that displayed four enzyme-like activities, oxidase, peroxidase, catalase, and superoxide dismutase.(Fan et al., 2018) By chemically conjugating the nanospheres with hollow human H-ferritin nanoparticle cores (HFn), which could specifically bind with HFn receptor transferrin receptor 1 (TFR1), they (HFn-N-PCNSs) were successfully guided to tumor cells that expressed a high level of TFR1 and localized to lysosomes (Figure 1di and Figure 1dii). Due to the favorable oxidase and peroxidase-like activities of the nanospheres in acidic microenvironment, ROS levels burst within the target tumor cells, which resulted in the significant tumor regression (Figure 1diii). Qiu et al. prepared a Cu²⁺-aspartic acid based supramolecular hydrogel with intrinsic peroxidase-like activity, which could catalyze low levels of H₂O₂ into ROS and inhibit the growth of bacteria that are responsible for wound infection. Meanwhile, Cu²⁺ slowly released from the hydrogel could stimulate collagen deposition and angiogenesis to accelerate the wound healing process. Combining the antimicrobial activity with tissue
repair capacity, a wound dressing prepared from the hydrogel showed remarkable wound healing properties in vivo.

**FIGURE 1** Artificial enzymes. (a) (i) Designing strategy of the ArM for the fluorescence turn-on detection of ethylene; (ii) Fluorescence imaging of a kiwi fruit with the ArM in three selected sections during the ripening process. (b) (i) Chemical structures of the block copolymer P1 and EUK; (ii) Schematic illustration of the internalization of the artificial organelles; (iii) Cell viability of HepG2 cells loaded with artificial organelle after stressed with paraquat after 24 h. The data are expressed as mean ± SD (n = 3, *p < 0.05, **p < 0.01). (c) (i) Schematic illustration of the molecularly imprinting TMB binding pockets; (ii) Photographs showing the selectivity differences for TMB with and without treatment of molecular imprinted polymers. (d) (i) Schematic illustration of N-PCNSs induced tumor cell destruction; (ii) TEM image of cancer cells treated with HFn-N-PCNSs. Red arrows indicate the location of HFn-N-PCNSs. Scale bar = 500 nm; (iii) Photographs showing the time-dependent evolution and progress of human HepG2 tumor morphology after treatment with HFn-N-PCNSs-3 (3 indicates a high doping level of nitrogen). Panel a adapted from Vong et al., (2019). Copyright 2019 Springer Nature. Panel b adapted with permission from Ade et al., (2019). Copyright 2019 American Chemical Society. Panel c adapted with permission from Zhang et al., (2017). Copyright 2017 American Chemical Society. Panel d adapted from Fan et al., (2018). Copyright 2018 Springer Nature

Designing AEs with natural enzyme-like activity is the first yet pivotal step toward cell mimicry. As discussed above, enormous progress has been achieved in the past few years. Initially starting from designing and synthesizing molecules/particles with enzymatic activities, the research interest in AEs has shifted a lot toward employing AEs for biomedical purposes in recent years. As the application of AEs for biomedical purposes is still in its infancy, several critical issues need to be addressed to reach the full potential of this new class of biomaterials, which will be discussed in more details in the penultimate section.
3. ARTIFICIAL ORGANELLES

Organelles are membrane-coated vesicles that perform a wide range of specific tasks inside (mammalian) cells. The segregation of membrane allows the organelles to perform different tasks in parallel without uncontrolled cross-reactions and undesired interference. Equipping cells with AOs can substitute for missing or lost cellular functions as well as non-native activity. AOs are typically envisioned as catalytically active intracellular nanoreactors, namely, single compartment nano-sized particles (10-300 nm) loaded with natural enzymes or AEs. The developments of AOs were discussed in several comprehensive reviews. (Peters et al., 2012; Garni et al., 2016; Itel et al., 2017) Here, we only aim to provide a focused overview of the advances in the last 3-4 years that considered AOs of sizes not exceeding 300-400 nm.

3.1 Carrier types

A majority of AOs utilize lipids or polymers as the main building blocks of the carriers presumably due to the ease of self-assembly and the diverse encapsulation capabilities. Lipid vesicles (liposomes) are a widely used, nature-borrowed concept that typically utilizes phosphatidylcholine lipids to form a bilayer surrounded aqueous void, similar to the cell membrane. There are only a few reports of liposome-base AOs,(Tiefenboeck et al., 2017), likely due to the fact that lipid vesicles can have stability issues when long-term (> 48 h) applications are envisioned. Alternatively, polymer–lipid hybrid vesicles (HVs) consist of a phospholipid bilayer in which amphiphilic block copolymers are incorporated, i.e., the hydrophobic part of the polymer is inserted into the phospholipid bilayer and the hydrophilic part ideally forms a “brush” layer surrounding the assembly (Schulz et al., 2015). Literature on HVs is relatively scarce in general with only one single report in the context of AOs (Zhang et al., 2019). Finally, polymersomes made from amphiphilic di- or tri block copolymer as a mimic of lipids also form a bilayer structure. Polymersomes have been quite popular scaffolds for AOs,(Discher et al., 2007) typically focusing on using poly(ethylene glycol) (PEG) as the hydrophilic block and poly(dimethyl siloxane), polystyrene, or polybutadiene as the hydrophobic block. (Nishimura et al., 2017; Einfalt et al., 2018; Einfalt et al., 2020) Polymers were also used for the assembly of micellar carriers,(Ade et al., 2019) cross-linked dispersed nanoparticles,(Huang et al., 2017) or as single chains.(Chen et al., 2020) In addition, inorganic materials, such as silica particles(Chen et al., 2018) and gold nanoparticles,(Zhao et al., 2018) were also successfully employed in the context of AO assembly.

3.2 Cytosolic Placement

There is an increasing number of reports on AOs being employed in biological context, not only in immortalized cells, but also in human derived primary cells and in animal models. Various core properties, such as cytosolic placement, heredity pattern, cytotoxicity as well as accumulation in organs after intravenous injection were considered. For instance, cytosolic placement of AOs is an important aspect since many targeted cellular functions occur in the cytosol and not the lysosomes/endosomes. Furthermore, in contrast to applications of nanosized particles for drug delivery purposes in which only the cargo is of interest (Ke et al., 2019; Mukerabigwi et al., 2019), AOs, as their name suggests, require that both the carrier and the encapsulated active entity remain intact upon cytosolic placement. In this section, examples of AOs that were successfully placed in the cytosol are discussed.
An interesting example was reported by Tiefenboeck et al. (Tiefenboeck et al., 2017) The authors showed that liposomes with and without PEGylation were stable in the cytosol of HeLa cells after microinjection. The inheritance pattern upon cell division was dependent on the size of the vesicles, that is, small (75 nm) liposomes passed on equally to daughter cells while larger (150 nm) liposomes were diluted nonlinearly. However, if the AOs are internalized via endocytosis, cytosolic placement, that is, endo/lysosomal escape, of the AOs remains a challenge. As for regular AOs, after their uptake by the cells (Zhao et al., 2018) they proceed to the endo/lysosomal system and then either being (partly) released into the cytosol or exocytosed. The process is accompanied by a pH change from 7.4 to around 4.5-6.0 during the maturation of the endosomes. The latter may pose a problem for pH sensitive compounds as they, for example, enzymes, could lose their activity. On the other hand, carriers can be engineered to take advantage of the pH drop, for example, to help the AOs to escape the endo/lysosomes avoiding digestion or exocytosis. Popular polymers used in this context contain tertiary amines, such as poly(2-[dimethylamino]ethyl methacrylate) (PDMAEMA) or poly(ethylene imine), which become protonated at lower pH, such as in the endosomes, and the content of the organelle escapes to the cytosol due to the bursting of the organelle’s membrane via the so called “proton sponge effect” (Selby et al., 2017) While the approach to place therapeutic cargo into the cytosol is highly abundant in nanomedicine, (Madani et al., 2011; Selby et al., 2017) it is a rarely employed strategy in the context of AOs due to their inherent toxicity (Zong et al., 2018; Ade et al., 2019) For instance, HVs consisting of poly(cholesterol methacrylate)-block-PDMAEMA (PCMA-PDMAEMA) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) were demonstrated to produce H$_2$O$_2$ and nitric oxide in RAW264.7 mouse macrophages when loaded with glucose oxidase and β-galactosidase (β-Gal), respectively (Zhang et al., 2019) Furthermore, human peripheral blood mononuclear cells from four donors were isolated and differentiated into human macrophages. In these cells, significantly higher nitric oxide levels were observed when treated with β-Gal containing AOs in the presence of the substrate compared to cells with empty AOs or substrate free environment (Figure 2a).

As an alternative to pH-responsive polymers, cell-penetrating peptides, in particular transactivating transcriptional activator (TAT) peptides, were considered to facilitate cytosolic placement of AOs (van Dongen et al., 2010) A recent example employed biodegradable and semi-permeable poly(ethylene glycol)-block-poly(caprolactone-gradient-trimethylene carbonate) (PEG-PCLgTMC) polymersomes loaded with catalase and equipped with TAT proteins for this purpose (van Oppen et al., 2018) The authors illustrated the ability of the AOs to protect patient-derived human-complex-I-deficient primary fibroblasts against the toxicity of exogenous H$_2$O$_2$ by utilizing bacteria derived catalase (Figure 2b). TAT peptides were also used to assist the cytosolic placement of GC-rich double-stranded oligonucleotides bound to nanogold (Au-ODN) to capture doxorubicin and protect noncancerous cells from the damage it caused. (Zhao et al., 2018) The assembly in human normal cells (QSG-7701 cells) had no toxicity and the formulation was protective. In mice, a high amount of Au-ODN was found in the liver that helped to reduce the side effects of doxorubicin and improved the well-being of the animals. It should be noted that this assembly is not a typical AO as it can be exhausted rather than performing over an extended period. Another relevant example is carbohydrate-block-poly(propylene glycol) CAPsoms-based AOs with encapsulated β-Gal (Nishimura et al., 2017) TAT peptide-modified β-Gal@TATCAPsoms acted as AOs in HeLa cells since they converted a nontoxic galactose linked substrate into cytotoxic doxorubicin.

3.3 Permeability
An essential property of AOs is their permeability, that is, substrates and products should easily pass across membranes while the encapsulated enzymes are retained. Well-organized lipid membranes as well as polymersomes have inherently very low permeability for many molecules, obstructing reactions in the void of the vesicles. (Baumann et al., 2017) The permeability of polymer-based nanoparticles were discussed in several comprehensive reviews. (Larrañaga et al., 2017; Belluati et al., 2019; Che et al., 2019) A recent highlight employed polymersomes, prepared from the above-mentioned biodegradable PEG-PClSiTMC, that exhibited sufficient permeability for H₂O₂ to reach the encapsulated catalase. (van Oppen et al., 2018) The carriers with encapsulated horseradish peroxidase (HRP) showed comparable $v_{max}$ values to free enzymes. However, a 70% increase in the $K_m$ values were observed, probably due to hindered diffusion of the substrates. As the above-mentioned reviews pointed out, several successful attempts were achieved by incorporating membrane proteins into the membrane of polymersomes. A very successful application is the insertion of the outer membrane protein F (OmpF) into the bilayer of polymersomes made of poly(2-methyloxazoline)-block-poly(dimethylsiloxane)-block-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) during the self-assembly process. (Einfalt et al., 2018; Garni et al., 2018) The polymersomes were loaded with HRP, and their ability to convert H₂O₂ was proven by the generation of the resorufin-like product. An elegant strategy to yield a compartmentalized system with complex composition was achieved by the generation of cell-derived giant plasma membrane vesicles (GPMVs) via blebbing caused membrane separation. (Einfalt et al., 2020) PMOXA-PDMSx-PMOXAy polymersomes were successfully incorporated into these GPMVs after endocytosis in HepG2 cells, creating subcompartmentalized artificial cells with a size of 5-10 μm. The ability to convert H₂O₂ was retained when OmpF was present in the assemblies both in vitro and in vivo after being deposited in zebrafish vasculature.

Numerous studies of nanoreactors in noncellular environments contributed to the understanding of the composition criteria and gave desirable guidelines for the design of AOs with efficient functionality in the intracellular environment. The permeability of phospholipid membranes can be increased by using polyunsaturated lipids over non- or mono-saturated lipids as illustrated by Nagatomo and Yoshimoto (Nagatomo et al., 2019). They evaluated the performance of encapsulated D-amino acid oxidase and found 4x better efficiency with higher unsaturation at 50 °C. HVs composed of various mixing ratios of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and poly(ethylene oxide-block-butadien) (EO₂₂Bd₃₇) were assessed with regard to their capability to incorporate ionophores, such as valinomycin, nigericin, and germicin, depending on the polymer content. pH sensitive dyes encapsulated inside of the HVs allowed for real-time monitoring of the pH change upon addition of a KOH solution, which increased the pH from 7.2 to 9.5 (Paxton et al., 2017). HVs showed higher permeability than the pristine vesicles made of only DOPC or EO₂₂Bd₃₇. Alternatively, the permeability of nanoreactors can be tuned by incorporating stimuli-responsive polymers in their membrane. Variation in the hydrodynamic diameter was achieved by using pH-responsive PDEAEMA (Che et al., 2018) and oligo(aspartic acid) (Nishimura et al., 2020) or temperature responsive poly(N-isopropyl acrylamide) (PNIPAM) in polymersomes. The ability of substrates and products to cross the membrane was dependent on the applied stimuli. Furthermore, incorporation of cross-linking groups, for example, epoxy, (Varlas et al., 2019) was shown to modulate permeability, resulting in over 80% reduction compared to nonmodified polymersomes, and the modulation was largely dependent on the size and hydrophobicity of the cross-linking molecules used.

3.4 Functionality
A majority of AOs utilizes enzymes as their active entities. The enzyme β-Gal was used in many functional AOs due to its high tolerance toward small chemical modification of its substrates. Several types of nontoxic substrates can be designed to release the product of interest, i.e., release of model drugs, (Chen et al., 2020) doxorubicin, (Nishimura et al., 2017) and nitric oxide, (Zhang et al., 2019). The first one is a recent example utilizing concurrent and cascade reaction of β-Gal and a Ru²⁺ complex in HeLa cells. First, the metal ion complex cross-linked single polymer chain particles (RuSCNP) aided the cytosolic placement of the free enzyme. Then, the RuSCNP was able to produce rhodamine while the enzyme freed coumarin from their respective quenched forms, or the RuSCNP activated the pre-substrate for subsequent reaction by the enzyme, producing fluorescently active molecules (Figure 2c). Reducing intracellular ROS levels has been an actively explored aspect for using AOs, (Garni et al., 2016) Recent examples include the use of horseradish peroxidase, (Chen et al., 2018; Einfalt et al., 2018) catalase, (van Oppen et al., 2018) and the catalase-mimicking EUK (Ade et al., 2019) as active entities in AOs toward ROS scavenging in HeLa cells, Raw 247.6 macrophages, human derived skin fibroblast, and HepG2 cells. In addition to the above mentioned doxorubicin-scavenging AOs, (Zhao et al., 2018) polydopamine nanoparticles were used as artificial microparasols that exhibit UV protection in HEKa cells, (Huang et al., 2017)

As a class of engineered nanoreactors, AOs are an indispensable part of engineering ACs. Several factors need to be taken into consideration when assembling an active AO, such as the type of enzymes to be encapsulated, the carrier type, the permeability of the carrier, intracellular fate after uptake, and so on. Each of these factors could have a significant impact on the application of AOs for biomedical purposes.
FIGURE 2 Artificial organelles. (a) (i) Cartoon illustrating the cytosolic placed nanoreactors loaded with glucose oxidase or β-galactosidase; (ii) NO production in primary human macrophages exposed to β-Gal-NONOate. Cell mean fluorescence (CMF) originated from the interaction with 4-amino-5-methyl-amin o-2,7-difluorofluorescein diacetate and intracellular produced NO. Data represent mean ± SD (**p < 0.01). (b) (i) PEG−PCl gTMC polymersomes loaded with enzymatic cargo and surface functionalized with cell-penetrating peptide (CPP); (ii) Functional analysis using primary skin fibroblasts from a healthy individual (C5120) and a patient with isolated complex I deficiency (S7-5175). Intracellular ROS assessed by chloromethyl-2,7-dichlorodihydrofluorescein diacetate fluorescence intensity measurements when cells retreated with polymersomes were challenged with H₂O₂ for 5 min. (c) (i) Illustration of RuSCNP-enzyme co-delivery and dual catalysis; (ii) Illustration of SCNP-enzyme tandem reaction conducted with RuSCNP and βGal. Flow-cytometry analysis of E. coli cells conducted with/without 8, RuSCNP, βGal, and irradiation. Panel a adapted from Zhang et al., (2019). Copyright 2019 the Royal Society of Chemistry. Panel b adapted from van Oppen et al., (2018). Copyright 2018 American Chemical Society. Panel c adapted with permission from Chen et al., (2020). Copyright 2020 American Chemical Society

4. ARTIFICIAL CELLS
Natural cells can be perceived as highly complex and advanced microreactors with a large number of specific functions. The key characteristics of living cells include compartmentalization, energy transduction and metabolism, growth, replication, communication and adaptability. (Buddingh et al., 2017; Yewdall et al., 2018) Integrating all these characteristics in a single synthetic system is a yet unachieved and ambitious goal. Synthetic systems that aim to mimic selected cellular activity are termed artificial cells (ACs), which are constructed by creating a structure from natural and synthetic building blocks in a bottom-up approach or by genetically engineering and/or encapsulating living cells in a top-down manner. (Xu et al., 2016; Farina et al., 2019; Kojima et al., 2019) We will only focus on the former aspect in this featured article. The purpose of ACs ranges from studying the origin of life with protocells and minimal cells (Stano et al., 2015) over developing novel AC-based therapeutics with the aim to support cells with missing/lost function (Itel et al., 2017) or to integrate nonnative activity on tissue level. The structural complexity of an AC can vary from simple single-compartment giant unilamellar vesicles (GUVs) to more complex, multicompartmentalized systems with a higher degree of hierarchical order. (de Hoog et al., 2012) Different concepts are used for constructing cell-sized compartments, such as polymersomes, (Meng et al., 2005) vesosomes, (Deng et al., 2017) GUVs, (Gopfrich et al., 2019) microcapsules, (Lu et al., 2017) microfluidic systems, (Gopfrich et al., 2018; Weiss et al., 2018) hydrogels, (Tan et al., 2017) coacervates, (Koga et al., 2011) capsosomes, (Städler et al., 2009) proteinosomes, (Liu et al., 2016; Qiao et al., 2017; Wang et al., 2019) etc. The envisioned property of the ACs often encompasses a single cellular function rather than mimicking the whole complexity of a biological cell. In the following section, we will highlight the progress in mimicking the compartmentalization with encapsulated (catalytic) activity, communication, and energy transduction.

### 4.1 Compartmentalization

Compartmentalization is essential for cellular function as it serves to create a boundary between different chemical milieus and maintain homeostasis under nonequilibrium circumstances. It facilitates different intracellular environments within the different organelles and allows for the whole cell to retain vital components while exchanging nutrients and waste byproducts with the surrounding environment. A simple example of a cell-sized semipermeable boundary that closely resembles the cell membrane is found in the form of GUVs, which was produced by a one-pot method and equipped with different functionalities, such as the reconstitution of integrin as well as encapsulation of enzymes, mitochondria or bacterial cells. (Gopfrich et al., 2019) The permeability of the separating membrane itself depends on the physiochemical properties of the amphiphilic building blocks, which can be phospholipids, (Bhattacharya et al., 2019) polypeptides, (Vogele et al., 2018; Schreiber et al., 2019) and block copolymers. (Meng et al., 2005) In addition, a higher degree of control over secretion and interaction with the buffer environment was demonstrated. (Hilburger et al., 2019; Debnath et al., 2020) Facilitation of spatially separated multi-step enzymatic pathways in separate compartments (Elani et al., 2014) and incorporation of an endomembrane system (internal membrane structures within a cell-sized carrier) to mimic the hierarchical structure of eukaryotic cells is the next step toward mimicking the architecture of a natural cell. (Deng et al., 2016; Tan et al., 2017; Gopfrich et al., 2018)

Compartmentalization can also be driven by liquid-liquid phase separation (LLPS) to create non-membrane bound organelles, as reviewed elsewhere. (Crowe et al., 2018; Ma et al., 2020) LLPS serves as a means to mimic organization of cellular fluids, as seen in e.g. nucleoli and cytoplasmic structures, (Godoy-Gallardo et al., 2017) and the condensates address the crowded intracellular
environment of cells. (Belluati et al., 2020). Polymer stabilized coacervates were used to mimic the cytosolic crowding (Yewdall et al., 2019) and coacervate systems were encapsulated into liposomes, which allowed for spatial organization of transcription. (Deng et al., 2017) Multicompartmentalized ACs that could act as cytosol-mimic were further demonstrated using membranized coacervates encapsulating polymersomal AOs. (Mason et al., 2019)

A step toward regulatory control over communication between compartments was shown using multicompartmentalized vesosomes with size-selective transfer of fluorescent dyes through incorporated protein pores between inner and outer compartments. (Deng et al., 2017) Lu et al. focused on constructing distinct internal compartments with different functions to more closely mimic the diversity of organelles using multicompartment capsules (MCC) made of biopolymers, such as chitosan and alginate. (Lu et al., 2017) The facilitation of a cascade process was shown by cultivating two strains of genetically engineered Escherichia coli in separate subcompartments of the MCC where one strain produced autoinducer 2 (AI-2), and the other acted as an AI-2 reporter that created a fluorescent protein in response to AI-2. A major challenge encountered when constructing ACs of higher complexity is the lack of control over the composition of encapsulated components during self-assembly and the limited possibility to manipulate the AC composition following the self-assembly. (Elani 2016) To address this issue, Weiss et al. constructed droplet-stabilized GUVs (dsGUVs) in a microfluidics device that allowed sequential loading of the dsGUVs with cytoskeletal components and transmembrane proteins by pico-injection. (Weiss et al., 2018) The stabilizing oil phase and droplet shells were subsequently removed to release the functionalized self-supportive ACs into an aqueous phase.

4.2 Encapsulated catalysis and therapeutic potential

Similar to how organelles carry out given tasks in a cell, the functions of an AC often depend on the encapsulated components in the cell-like assembly. Constructing ACs can be done by employing either a top-down or bottom-up approach. Cell encapsulation is a central concept in top-down assemblies, where microbeads are used to provide a protective and supportive environment for cells to perform the therapeutic effect, as reviewed elsewhere. (Farina et al., 2019) Catalytic activity can be introduced using enzymes, (Itel et al., 2017) enzyme mimics, (Armada-Moreira et al., 2018) and enzymatic cascades, (Elani et al., 2014; Fujiwara et al., 2018) as encapsulated cargo in bottom-up assembled cell-sized compartments.

Another popular approach to provide AC functionality is by utilizing cell-free expression systems within cell-sized compartments, as reviewed in detail elsewhere. (Jeong et al., 2019; Yue et al., 2019; Lyu et al., 2020; Noireaux et al., 2020) Incorporating the machinery for protein transcription-translation (TX-TL) allows for the AC to contain information in a similar manner to the natural cells by utilizing nucleic acids (DNA and RNA) as information carrying molecules and serve as a means to program and develop functions in the AC. Cell-free gene expression within micro-sized vesicles was previously demonstrated. (Nomura et al., 2003; Noireaux et al., 2004; Shin et al., 2012) This method has recently been utilized to insert expressed membrane proteins into a lipid carrier membrane, (Ho et al., 2015) and the execution of five- and six-gene cascading circuits within liposomal vesicles were achieved. (Garamella et al., 2016) Hydrogel based organelles were further implemented to achieve compartmentalization with spatial separation of transcription and translation. (Aufinger et al., 2018) Progress also involves the demonstration of complex behaviour in cell-like carriers, such as artificial
immune response programmed by DNA reaction network,(Lyu et al., 2018) communication,(Adamala et al., 2017; Joesaar et al., 2019) and implementation of a transcriptional oscillator system(Weitz et al., 2014). In addition, with the aim to benefit from the biological diversity and complexity, co-encapsulation of natural and synthetic organelles was demonstrated using purified nuclei and catalase-loaded liposomes to create multicompartmentalized microreactors.(Zhu et al., 2018) Intact cells were encapsulated in a vesicle-based AC as an organelle-like module working in concert with a synthetic co-encapsulated enzymatic cascade to provide more complex functions to the construct than what is currently achievable with synthetic subunits only.(Elani et al., 2018)

Thus far, the interaction of ACs with their natural counterparts remains underexplored. However, this is of essential importance when using ACs for biomedical applications. ACs hold promise as an alternative therapeutic approach, where the ACs exhibit beneficial native or non-native function of their mammalian cell counterpart to supply missing cellular function. First examples of AC interactions with natural cells include targeting pathways of Escherichia coli for sensory expansion(Lentini et al., 2014), functionalizing microparticles with stem-cell factors and membrane for injection in mice with myocardial infarction(Tang et al., 2017) and H$_2$O$_2$ and ammonia depletion using microreactors equipped with platinum nanoparticles in neuroblastoma cell cultures(Armada-Moreira et al., 2018). The effect of ACs on hepatocytes in 2D cell cultures(Zhang et al., 2017) or in cell aggregates(Zhang et al., 2017) was explored for cytotoxic H$_2$O$_2$ removal using catalase-loaded liposomes as subunits. ACs were explored as alternative cancer therapeutics by developing lipid vesicles producing anti-cancer proteins inside tumors(Krinsky et al., 2018), or by constructing tyrosine-kinase expressing microreactors for depletion of melanoma cells(Godoy-Gallardo et al., 2019). Additionally, a composite scaffold system that mimics natural antigen-presenting cells was constructed to stimulate ex vivo T-cell expansion, where a supported lipid bilayer on mesoporous silica micro-rods serve to present membrane-bound T-cell activation cues.(Cheung et al., 2018)

Further, matrix vesicle-containing microreactors were developed by Itel et al.(Itel et al., 2018) with the aim to enhance biomineralization properties of osteoblasts at the site of bone fractures and thereby potentially aid the natural healing process. Specifically, functional microreactors made from alginate beads loaded with artificial or purified matrix vesicles (MVs) were co-assembled with osteoblast-like SaOS-2 cells to form 3D spheroids (Figure 3ai). The presence of the MVs would thereby serve to enhance the biomineralization properties of the osteoblast-like cells (Figure 3a(ii)) and enhance cellular function in presence of the microreactors.

4.3 Communication

Cells are most often found in groups where the use of chemical signals for communication is ubiquitous and serves to coordinate the collective behavior of the cellular assemblies. Managing communication and information processing can be done in different ways. ACs created through top-down engineering of mammalian cells to control cell-cell communication were reviewed elsewhere.(Kojima et al., 2019) In bottom-up approaches, a popular method is to use synthetic gene circuits benefiting from the programmable and modular nature.(Adamala et al., 2017; Tayar et al., 2017; Dwidar et al., 2019; Rampioni et al., 2019)

Communication and interaction can be considered between ACs and the surrounding aqueous environment,(Zhuang et al., 2019) biological cells(Lentini et al., 2017; Rampioni et al., 2018; Wang et
al., 2020) or other AC populations (Qiao et al., 2017; Ding et al., 2018; Rodriguez-Arco et al., 2019). Responsiveness to the surrounding environment, where an incoming signal leads to a functional change in behavior, is vital toward the development of an adaptive AC. A recent example of an AC showing adaptive behaviour in response to environmental stresses was reported by using DNA nanotechnology to construct a cyclic feedback network on GUVs which could sense, respond and eliminate incoming stimuli through DNA strand-displacement reactions (Liu et al., 2019) Employing mechanosensing to mimic cell communication through a de novo signalling pathway (Hindley et al., 2019) and for biosensing (Majumder et al., 2017) is another interesting progress in the development of ACs. Garamella et al. developed an mechanosensing AC equipped with an inducible genetic AND gate that, in the presence of the isopropyl β-D-1-thiogalactopyranoside inducer and hypooosmotic conditions, express E. coli cytoskeleton protein MreB that associates with the inner membrane of liposomes to create a cytoskeleton cortex (Garamella et al., 2019) Alternatively, communication between biological cells and ACs was illustrated using GUVs and red blood cells, which interacted through an enzymatic cascade reaction under spatial organization using acoustic trapping (Wang et al., 2020) An example of AC-AC communication was shown with ACs made from a porous polymer membrane containing a clay-DNA-hydrogel as a nucleus mimic to achieve quorum sensing in a large population of ACs (Niederholtmeyer et al., 2018) Two AC types were made each containing part of a two-stage activation cascade involving T3 RNA polymerase (T3 RNAP) as the diffusive signalling protein and TetR-sfGFP as the fluorescence reporter gene to show the exchange of proteins and communication through genetic modulators (Figure 3bi). Another AC containing both activator circuit and reporter constructs were made (Figure 3bii) to serve as a reporter of AC population density, where fluorescence only accumulated at higher cell densities (Figure 3biii), similar to bacterial quorum sensing.

4.4 Energy transduction

When aiming to create self-sustaining ACs under nonequilibrium conditions or to facilitate a wider array of encapsulated endergonic functions, a working metabolism to sustain biomimetic processes is of importance. Supplying the AC with its own source of energy is a vital step forward to this end. The recent progress in generating energy carriers in AOs was discussed in detail by Otrin et al. (Otrin et al., 2019) while the implementation of energy carriers in AC systems are scarce. An example of photophosphorylation using a light-induced proton gradient inside an AC was reported by Berhanu, Ueda, and kuruma (Berhanu et al., 2019). They combined cell-free protein synthesis (PURE system) with proteoliposomes (PLs) containing ATP synthase and bacteriorhodopsin (bR) inside GUVs to drive photosynthesis of adenosine triphosphate (ATP), where the produced ATP was used as a substrate for transcription and energy carrier to facilitate translation (Figure 3ci). An AC containing the PL and translation-only PURE system was used to drive the de novo production of component proteins of the PL (Figure 3cii), bR (Figure 3ciii), and the ATP synthase subunit F0. The proteins could integrate into the artificial photosynthetic organelle in a positive feedback loop, which led to an increase in ATP production (Figure 3civ) due to an increase in bR and F0 being produced and incorporated in the PLs. This is a relevant step toward making a self-sustaining system as utilizing bR provides access to an unlimited external energy source, light. Further, a photosynthetic AO based on two photoconverters was previously used by Lee et al. to demonstrate actin polymerization inside GUVs (Lee et al., 2018) Recently, Miller et al. constructed a chloroplast mimic by co-encapsulating thylakoid membranes from spinach with a synthetic enzymatic cycle in cell-sized droplets (Miller et al., 2020) It should be noted
that the very limited examples of ATP production in a cell-like carrier had thus far focused on photosynthetic rather than chemically driven ATP-production.

In an effort by Pols et al., sustained ATP levels using a metabolic network in proteoliposomes were reported (Pols et al., 2019). ATP production under the metabolic conversion of L-arginine to L-ornithine was kept from reaching thermodynamic equilibrium by using membrane-embedded antiporters. It was demonstrated that upon co-reconstitution of the metabolic network with the ATP-driven glycine betaine transporter OpuA, the balance of osmolytes in the vesicles could be modulated. Increasing the surrounding buffer osmolarity resulted in an increase in internal ionic strength, which activates OpuA, leading to ATP consumption upon gated transport of glycine betaine across the membrane. This effort highlighted maintenance of the physicochemical homeostasis under nonequilibrium circumstances and showed cell-like metabolic energy conservation and volume regulation in nanosized compartments.

The construction of energy producing AOs and ACs often faces the challenge that there is a lack of coupling between recycling of redox cofactors (Lin et al., 2018; Wang et al., 2018), such as nicotinamide adenine dinucleotide (NAD+/NADP) and ATP production, which is necessary to create a truly self-sustainable system. An early example of coupled sodium driven ATP production and NAD+ regeneration was achieved using sodium ion cycling (Gemperli et al., 2003). More recently, microfluidics were used by Beneyton et al. (Beneyton et al., 2018) to construct water-in-oil droplets as microcompartments encapsulating a minimal metabolism consisting of NAD+-dependent enzymatic reaction and a NAD+-regeneration module using inverted membrane vesicles (IMVs) extracted from Escherichia coli.

4.5 Other features

Natural cells contain a plethora of components and functions that could be mimicked beyond the hierarchical architecture, encapsulated catalysis, communication, and ATP production. For instance, the focus can be on the complexity of the plasma membrane, (Tang et al., 2017; Toparlak et al., 2019) the cytosol, (Einfalt et al., 2020), the cytoskeleton, (Bashirzadeh et al., 2019) or the overall cellular morphology. (Litschel et al., 2018; Fanalista et al., 2019). Further, membrane expansion was achieved using a cascading biosynthesis pathway containing eight membrane proteins (Exterkate et al., 2018) and the self-reproduction of boundary membrane layers was recently reviewed elsewhere (Exterkate et al., 2019). The cytoskeleton has received attention to create dynamic ACs with actin polymerization inside GUVs, (Lee et al., 2018) artificial cilia using microtubule/kinesin (Sasaki et al., 2018) or nematic alignment of actin to study cell movement through repetitive motion upon external stimuli (Tanaka et al., 2018). Controlled deformation of GUVs was developed using 3D-printed protein hydrogel scaffolds to dynamically achieve spatial anisotropy under pH stimuli. (Jia et al., 2020) These progresses could serve to improve our understanding of cellular phenomena, such as division, differentiation, migration, and signalling that often depend on distinct cell morphologies. Liu et al. employed an alternative approach for AC construction by using coordination network materials. (Liu et al., 2019) Metal-phenolic network microcapsules with encapsulated enzyme-containing, biocatalytic metal-organic framework AOs were used for increased thermal and chemical stability as well as making the AC responsive to stimulus. Several cellular function features were mimicked such as enzymatic cascade reactions by encapsulating GOx and HRP-containing AOs in the same AC, communication by having GOx and HRP AOs encapsulated in two separate ACs and programmed proteolysis through co-encapsulation of AOs loaded with either trypsin or DQ–ovalbumin (which emit fluorescence upon trypsin proteolysis).
In addition, focus has been put on various other features, such as cell adhesion, (Bartelt et al., 2019) substrate uptake and processing, (Hansen et al., 2015) light-triggered pinocytosis, (Konetski et al., 2018) phagocytosis, (Nikolov et al., 2019) and predatory behaviour, (Qiao et al., 2017) Further, examples of adaptive behaviour was seen through differentiation along morphogen gradients. (Dupin et al., 2019) An example of mimicking cellular differentiation was done using a single population of immobilized coacervate micro-droplet arrays that encapsulate HRP. (Tian et al., 2019) These develop into a multimodal population of ACs exhibiting spatially dependent enzymatic activities due to morphogen-induced differences in vesicle membrane permeability.

ACs aim to mimic chosen properties of the complex natural (mammalian) cells, and progress has been seen toward mimicking several of these functions in recent years. Compartmentalization and hierarchical assembly are continuously an essential feature of interest, as well as the various ways of employing functions to the AC using a bottom-up strategy. The latter aspect is often accomplished by encapsulating cargo and enzymes, cell-free expression systems and biological subunits. Demonstrated functions include supplying therapeutic effects to natural cells through enzymatic conversions, mimicking communication via chemical signals or achieving a minimal metabolism with focus on ATP production toward self-sustaining ACs.
FIGURE 3 Artificial cells. (a) Encapsulated catalysis: (i) Schematic presentation of SaSO2 cells co-cultured with matrix vesicle (MV) loaded alginate beads to create spheroids; (ii) Relative total Ca²⁺-content quantified after 3, 7, and 14 days of incubation of spheroids in osteoconductive media consisting of cells only (S-C), empty alginate beads and cells (S-M⁰) or MV-loaded alginate beads and cells (S-MMV). (b) Communication: (i) Schematic of the activator and reporter cell pair. The activator cell contains the template for T3 RNA polymerase (T3 RNAP) expression. Reporter cells undergo T3 RNAP driven expression of a fluorescent fusion protein of the tetracycline repressor TetR and sfGFP (TetR-sfGFP) and the tet operator sequence (tetO) array plasmid to localize the TetR-sfGFP fluorescence to the hydrogel nucleus. Micrographs show a rhodamine B fluorescence in activator
membranes and TetR-sfGFP in the hydrogel nucleus of reporter cells; (ii) The artificial quorum sensing cell contain DNA templates for T3 activation cascade and tetO array plasmid which drives TetR-sfGFP production; (iii) Micrographs of ACs in droplets of cell-free transcription and translation (TX-TL) reagents with the number of cells is indicated (left panel). The enlarged regions shown in the right panel, indicated with the white box on left images, show green fluorescence after 3 h of incubation. (c) Energy transduction: (i) Overview of the AC; (ii) Schematic of the AC containing the PL and translation-only PURE system to facilitate bacteriorhodopsin (bR) and ATP synthase subunit F0 synthesis; (iii) Confocal laser scanning microscopy images of light-induced de novo bR-GFP production inside GUVs; (iv) Light-driven ATP synthesis by PLs with de novo F0 subunit synthesis with wild type (awt) or mutant (awt) subunit was synthesized. Panel a adapted with permission from Itel et al., (2018). Copyright 2018 American Chemical Society. Panel b adapted from Niederholtmeyer et al., (2018). Copyright 2018 Springer Nature. Panel c adapted from Berhanu et al., (2019). Copyright 2019 Springer Nature

5. Challenges and Perspectives

With the aim to mimic natural cells with a bottom-up strategy, the past few years have witnessed the substantial progress in the synthesis, assembly, and application of AEs, AOs, and ACs. There is no doubt that the results are fruitful, but in the meantime, we must admit that challenges still remain, and many critical issues need to tackled in this burgeoning field.

AEs are continuously striving to replace their natural counterparts with reinforced stability and tailor-made functionalities. However, they are not all-purpose tools so far. First, most AEs show rather low catalytic activity and substrate specificity compared to natural enzymes. Second, the choices of enzymes that had been mimicked are dominated by redox enzymes. Developing artificial synthases would be highly relevant. Third, most of the reported AEs have mono-functionality. In the case of catalyzing a cascade reaction, which requires the participation of multiple enzymes, compatibility of different AEs could be problematic as they commonly require different optimal working conditions. Last but not the least, most AEs are still at the proof-of-concept stage, problems like long-term stability/toxicity and controlled localization to the desired cells or tissues need to be solved before AEs can be widely employed to address biomedical challenges.

As for AOs, there is still only a limited number of reports where AOs were successfully implemented in the cellular environment. However, it is especially promising that functional AOs were reported not only in immortalized cell lines, but also in primarily human cells and animal models. There is no single optimal design to assemble functional AOs but several possibilities with regard to employed building blocks and functional entities are being explored. Advances in tuning the permeability of the carriers have been accomplished. However, it remains often trial-and-error to obtain sufficient transport of substrates and products across the membrane of the AOs. Further, controlled and successful cytosolic placement of AOs is still a long-standing challenge. Generalizable concepts and methods for the comparable verification of lysosomal escape remain to be identified. The structural integrity and the long-term fate (heredity pattern) of AOs is another aspect that requires attention. The functional diversity of intracellular active AOs is still very limited, often focusing on model substrates or ROS scavenging. AOs that can synthesize (therapeutic) molecules inside of cells would be a game-changer.

ACs have gained much attention as synthetic models to study the different functions and mechanisms of actions of their natural counterparts. However, the vast majority of ACs uses natural enzymes to introduce functions, which inherently limits the performance of the ACs. AEs could be envisioned to circumvent some of these challenges in particular the long-term stability. Further, a rather limited
number of enzymatic functions are employed and expanding the variety of functions could be dealt with by, such as combining top-down and bottom-up concepts, using natural organelles in synthetic cell systems, or supplying the ACs with their own source of energy to utilize for ATP-dependent enzymes and anabolic processes. Progress was made for the latter aspect toward designing systems capable of ATP production, but the development of chemically driven energy production remains underexplored. To this end, recycling of cofactors should also be addressed to improve the self-sustainability to realize fully autonomous ACs. Expanding the variety of enzymatic reactions to be employed in AC populations can further expand the types of communication studies, which often rely on model cascade reactions. An interesting opportunity could be to combine therapeutic effects of ACs in cell culture with communication capabilities, such that the AC response depends on the state of the cell culture. Reports involving communication of ACs are an interesting step forward toward creating more tissue-like organization of ACs. In addition, a better understanding of reaction–diffusion processes, cellular pattern formation and collective responses through large macromolecule signalling could be obtained. In this context, the hierarchical structure of multi-cellular organisms is a future prospect to be considered including varied mechanical properties and controlled mammalian cell–AC contact. Finally, the incorporation of multiple functional modules into the same AC to mimic more characteristics of a cell simultaneously needs to be considered in the future.

6. CONCLUSION

As a nature inspired concept, cell mimicry aims to integrate rationally designed molecules and hierarchically assembled nanomaterials with mammalian cells and eventually tissue, providing potential solutions to combat biomedical challenges. This feature article summarized the latest developments in the three core building blocks of cell mimicry: AE, AO, and AC. The key issues of each building block were outlined individually, and some of the novel examples were elaborated in detail. The challenges and opportunities in this emerging filed were highlighted in the end. Taken together, the overall developments are happening at an impressive pace. Artificial biology is therefore on the way to become a complementary field to nanomedicine and synthetic biology.

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